Sarcomeric Genes Involved in Reverse Remodeling of the Heart During Left Ventricular Assist Device Support

Amélie Rodrigue-Way, MS,[a] Daniel Burkhoff, MD, PhD,[b] Bard J. Geesaman, MD, PhD,[c] Serge Golden, BS,[a] Jian Xu, MD,[a] Matthew J. Pollman, MD,[a] Mary Donoghue, BS,[a] Raju Jeyaseelan, PhD,[a] Steven Houser, PhD,[d] Roger E. Breitbart, MD,[a] Andrew Marks, MD,[b] and Susan Acton, PhD[a]

Background: Left ventricular assist devices (LVADs) implanted in patients with severe congestive heart failure (CHF) as a bridge to transplantation have been shown to reverse chamber enlargement, regress cellular hypertrophy, and increase contractility. The purpose of this study was to gain a better understanding of the molecular changes associated with increased contractility after LVAD support.

Methods: We took tissue sections from the left ventricular apex of 12 patients with CHF who were undergoing LVAD insertion (pre-LVAD) and from the LV free wall of those same patients before transplantation (post-LVAD). To control for sample-site differences, we obtained samples from the same regions in 7 patients with CHF who were undergoing transplantation without LVAD support and in 4 non-failing donor hearts. Gene expression was then probed on a custom DNA array containing 2,700 cardiac-enriched cDNA clones.

Results: Calcium-handling genes were up-regulated by LVAD support, as previously reported. Sarcomeric genes were the other principle class of genes up-regulated by LVAD support, consistent with a possible restoration of sarcomere structure in reverse ventricular remodeling. However, a decrease in the fibrous component of the myocardium, also potentially involved in reverse remodeling, was not evident at the level of gene transcription because fibroblast markers were either unchanged or up-regulated. The remaining regulated genes did not fall into any defined functional class.

Conclusions: Changes in the regulation of sarcomeric, calcium-handling, and fibroblast genes during LVAD support indicate a cardiac molecular adaptation to mechanical unloading. These molecular changes may play a role in the observed increase in contractile function during reverse remodeling. J Heart Lung Transplant 2005;24:73–80. Copyright © 2005 by the International Society for Heart and Lung Transplantation.

Congestive heart failure (CHF) represents the final manifestation of a number of cardiovascular diseases, such as ischemic heart disease, hypertension, valvular lesions, primary cardiomyopathies, and viral infections. Cardiac remodeling occurs during progressive heart failure and involves adverse changes at the molecular, cellular, and interstitial level that result in the alteration of heart size, shape, and function. These changes include hypertrophy, chamber dilation, wall thinning, hypocontractile function, myofibrillar disarray, and increased fibrosis, all of which compromise cardiac function.

Reverse remodeling in turn is defined as a decrease in left ventricular (LV) chamber dimension and improved function.[1] During support of critically ill patients with left ventricular assist devices (LVADs), the decrease in ventricular pressure and volume load results in a reversal of chamber enlargement, normalization of cardiac structure,[1–3] and improved myocyte function.[4,5] Studies have shown a decrease in neuroendocrine activation during LVAD support, including a decrease in angiotensin II, plasma epinephrine, norepinephrine, and arginine vasopressin levels, and plasma renin activity.[6] Tumor necrosis factor-α protein, increased in heart failure, also is decreased in the myocardium after LVAD support.[7,8] Numerous other studies have reported down-regulation of natriuretic factors,[9,10] up-regulation of calcium-handling proteins,[5,11,12] reversal of the down-regulation of β-adrenergic receptors associated with heart failure,[13] and down-regulation of matrix metalloproteinases.[14] Because of the documented dramatic changes

From [a]Millennium Pharmaceuticals, Cambridge, Massachusetts; [b]Columbia University, College of Physicians and Surgeons, New York, New York; [c]Elixir Pharmaceuticals, Cambridge, Massachusetts; and [d]Temple University School of Medicine, Philadelphia, Pennsylvania.

Submitted June 10, 2003; revised October 15, 2003; accepted October 15, 2003.

This work was part of a joint program of Eli Lilly and Millennium Pharmaceuticals.

Reprint requests: Roger Breitbart, MD, Department of Cardiology, Children's Hospital Boston, 300 Longwood Avenue, Boston, Massachusetts 02115. Telephone: 617-355-2731. Fax: 617-739-3784. E-mail: roger.breitbart@cardio.chboston.org

 1053-2498/05/$–see front matter. doi:10.1016/j.healun.2003.10.016

in cardiac geometry and the increase in contractile function after LVAD support,[4,5] we hypothesized that additional transcriptional changes would participate in this reverse-remodeling process.

METHODS

Heart and Tissue Harvest

Under protocols approved by the institutional review boards of the New York Presbyterian Hospital and Temple University, we used for this study LV myocardium obtained from 19 patients with end-stage heart failure (CHF) and myocardium from 4 non-failing donor hearts. Of the CHF hearts, 12 had been supported with LVADs for an average of 59.7 ± 36.1 (range, 17–120) days. The non-failing hearts were unsuitable for transplantation for various reasons unrelated to hypertrophy. For patients with LVAD support, insertion and operation of the devices (Heartmate I, Thoratec; Woburn, MA) was performed as previously described.[15] We excluded regions of apparent infarct during sampling. Inflow to the LVAD is provided through a conduit inserted into the LV through an ~1-inch hole made in the apex. All samples were taken transmurally so that endocardial and epicardial differences would not contribute to the differences seen here. Tissue removed in making this hole was immediately frozen in liquid nitrogen and subsequently used for "pre-LVAD" analyses. At the time of transplantation, all hearts were perfused with 4°C hypocalcemic, hyperkalemic cardioplegia solution. Tissue taken after LVAD support and removal of the heart for transplantation was from the LV free wall (post-LVAD sample). To determine potential regional differences in gene expression unrelated to LVAD support, we analyzed free-wall and apex samples for gene expression levels from patients with CHF who were undergoing transplantation without LVAD support. In all cases, we froze tissue samples in liquid nitrogen immediately after excision. Normal human left ventricular cardiomyocytes and cardiofibroblasts were isolated as previously described.[16] Total RNA was extracted using RNA-STAT (Tel-test; Friendswood, TX).

cDNA Array Construction, Hybridization, and Data Analysis

Bacterial cultures of 2,700 cDNA pre-selected clones from 2 heart libraries, 1 heart subtracted library, various IMAGE clones (ResGen, an Invitrogen Corporation; Huntsville, AL), and 300 other relevant known gene clones were consolidated into 96-well plates. We performed custom array preparation, sequence verification, hybridization of labeled probes, and all subsequent data capture as previously described.[17] Briefly, ^{33}P-labeled cDNA was generated from total RNA[17] and hybridized to triplicate nylon arrays containing spotted polymerase-chain-reaction products. After hybridization and washing, we detected the signals using a phosphoimager and digitized signals using data-capture software. To normalize expression distributions across arrays, we transformed the data using a moving window that mean-normalized the expression levels within ranked bins of 100 clones. We used a permutation test that incorporated a global noise model to score each clone for statistical significance. To construct the global noise model, expression data were partitioned into ranked bins, based on the clone mean-expression level. We used a function relating expected variance to mean expression level as the global noise model. For each clone in each set of comparisons, the means of the samples compared were subtracted pair-wise. We determined within-group variance for each clone to model local noise of the data. To improve the accuracy of the noise estimation, we combined local and global expected variance estimates in a weighted function (0.8 and 0.2, respectively.) For each test, 100 random permutations (permuted across arrays) of the data were constructed for comparison with the observed data. We calculated the test statistic as the ratio of the difference of means and the combined noise estimates. For each of these 100 randomized data sets, a test statistic for each clone was computed, as was computed for the original data. For each clone, by comparing observed randomized data, we obtained false-positive rates as described elsewhere.[18] Pair-wise comparisons within the same patient were chosen to eliminate as many variables as possible. To determine fold change, we calculated the post/pre or free-wall/apex ratio for each patient and determined the median of all fold changes in each group. For genes that were represented by more than 1 clone, we report the median fold change and an adjusted p value.

To address the reproducibility of the results, we performed a transcription-profiling study of a smaller pre- and post-LVAD group and obtained results similar to the those presented here.

Slot Blot Northern Confirmation

We confirmed regulation using a slot blot Northern analysis. A dilution series of total RNA (1.8 μg–28 ng) was loaded directly onto a nylon membrane using a slot blot apparatus. The membrane was then pre-incubated with a sodium dodecylsulfate/Triton X-100–based nylon wash solution for 4 hours at 65°C and then hybridized overnight with a ^{33}P-labeled probe. After washing, the blot was exposed to a phosphoimager screen for 4 to 64 hours, depending on intensity detection levels, and then quantitated with a phosphoimager apparatus.

Table 1. Patient Characteristics, Hemodynamics, and Medication Use

	Medical support	LVAD Support	
	Transplant	Implant	Transplant
Number of patients	7	12	
Age	59.4 ± 6.1	54.0 ± 9.9	
Male/female	6/1	12/0	
ICM/DCM	6/1	8/4	
Duration of LVAD support (days)	N/A	59.7 ± 36.1	
Ejection fraction (%)	20 ± 9	13 ± 2†(n = 11)	N/A
Central venous pressure (mean, mm Hg)	6 ± 7	17 ± 5†	9 ± 5*
Pulmonary artery pressure (mean, mm Hg)	26 ± 13	41 ± 7†	22 ± 9*
Pulmonary artery diastolic pressure (mean, mm Hg)	18 ± 9	31 ± 7†	15 ± 7*
Mean arterial pressure (mm Hg)	80 ± 15	75 ± 9	83 ± 11
Cardiac output (liter/min)	3.7 ± 0.8	3.3 ± 0.7	3.8 ± 0.5*
Medications			
Inotrope (dobutamine/milrinone)	5 (3/4)	12 (10/8)	2 (1/1)
Dopamine	2	7	2
β-Blockers	3	2	1
ACE-Inhibitors	4	8	6
Diuretic	7	12	4
Aldactone	5	3	0
Digoxin	5	7	2
Amiodorone	0	4	3
Lidocaine	0	1	0
IABP	0	3	0

*$p < 0.05$ vs LVAD implantation; †$p < 0.05$ vs transplantation in medically supported transplant recipients.

LVAD, left ventricular assist device; N/A, not applicable; ICM, ischemic cardiomyopathy; DCM, idiopathic dilated cardiomyopathy; IABP, intraaortic balloon pump; ACE, angiotensin-converting enzyme.

The signal was normalized against the signal from the same blot hybridized with total heart cDNA probe. The specific signal was determined in the linear range of the curve.

RESULTS

To better understand the underlying molecular changes resulting in increased cardiac contractility during LVAD support, we examined gene-expression changes in samples from 12 patients receiving LVAD support. Table 1 shows demographic data. The cause of heart failure was predominantly ischemic heart disease, and the average length of support was 59.7 days, ranging from 17 to 120 days. Hemodynamic data were collected from all patients, under anesthesia, at the time of heart transplantation or LVAD insertion. Compared with patients undergoing primary transplantation, patients requiring LVAD support had decreased ejection fractions, increased right-sided pressures, and trends toward lower cardiac outputs and blood pressures at implantation. The LVAD support decreased central venous and pulmonary artery pressures, improved cardiac output, and was accompanied by marked decrease in the need for intravenous inotropic agents, dopamine, and diuretics. All patients with LVAD support received inotropic support at the time of implantation.

We used total RNAs from the samples to prepare cDNA probes that were then hybridized to a cardiac-enriched cDNA array containing 2,700 clones. Despite dramatic clinical changes (e.g., improved cardiac output), the largest observed change in gene expression was just 1.61-fold, $p < 0.001$ (post-/pre-LVAD) for FHL-2 (Four and a half LIM domains protein 2, Genbank #U29332). The gene that was the most transcriptionally down-regulated was lipoprotein lipase (Genbank #M15856, 0.63-fold, $p = 0.005$, post-/pre-LVAD). Overall, we found 211 clones to be regulated in LVAD support (≥20% change with $p \leq 0.05$).

To validate this experiment, we examined the expression of genes previously shown to be regulated by LVAD support. As expected, we noted a change in calcium-handling gene expression. Sarco(endo)plasmic Reticulum Ca^{2+}-ATPase mRNA was up-regulated by LVAD support (post-/pre- LVAD = 1.29-fold, $p < 0.0001$, by micro-array and 1.21-fold by slot blot Northern, data not shown), consistent with published reports.[5,11] Although the up-regulation of the ryanodine receptor 2 is small (post-/pre-LVAD = 1.10, $p < 0.001$, data not shown), its regulation also is consistent with previous reports.[5]

We observed a global increase in the mRNA levels of sarcomeric proteins as a result of LVAD support (Figure 1 and Table 2). For example, cardiac beta-myosin heavy

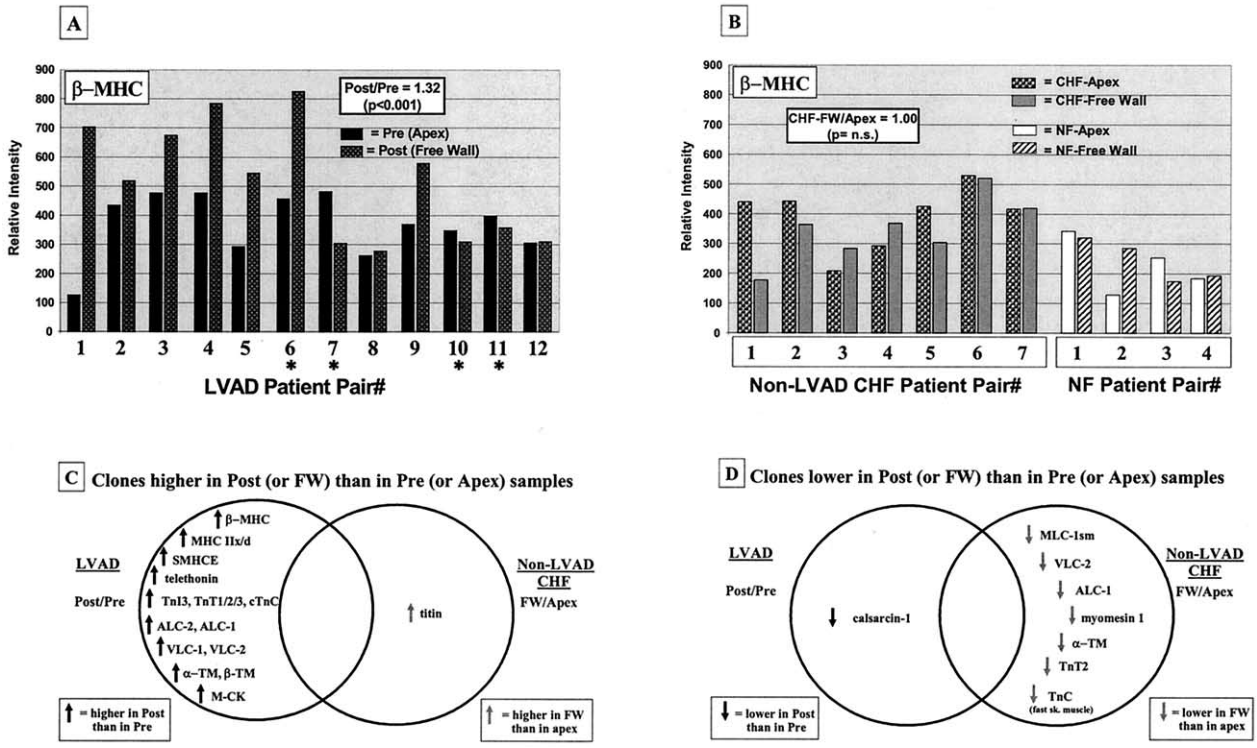

Figure 1. Sarcomeric gene expression differences in pre–left ventricular assist device (LVAD) and post-LVAD support and in non-LVAD congestive heart failure (CHF) samples. Distribution of the relative intensity levels of beta-myosin heavy chain (β-MHC) across individual paired patient samples (A, B). The graphs represent the relative intensity of a single clone. The fold change and the p value represent the median fold change and the adjusted p value for all clones on the array associated with this gene. Significant expression differences ($p < 0.05$) were separated into 2 groups according to their direction of change: C) genes expressed at greater levels in post-/pre-LVAD or at greater levels in free wall/apex in the non-LVAD CHF group, and D) genes expressed at lower levels in post-/pre-LVAD or at smaller levels in free wall/apex in the CHF group. Nebulette, MyBP-C, and skeletal and cardiac alpha-actin were not regulated by LVAD support. The following genes were not present on the array: troponin I isoforms TnI1 and 2, α-actinin, tropomodulin, capZ, and smooth muscle alpha-actin. *Samples from patients with dilated cardiomyopathy who received LVAD support. ALC, atrial embryonic myosin alkali light chain; FW, free wall; NF, non-failing; n.s., not significant; M-CK, creatine kinase M chain; post-, after LVAD support; pre-, before LVAD support; SMHCE, embryonic myosin heavy chain skeletal muscle; TM, tropomyosin; VLC, ventricular myosin alkali light chain.

chain (β-MHC) was 1.32-fold greater in post- vs pre-LVAD samples (14 independent elements on the array, $p < 0.001$, Figure 1A). Up-regulation of β-MHC expression also was detected in a 2nd independent-array experiment and confirmed by slot blot Northern analysis (1.27-fold, data not shown). Figure 1C and D shows 16 other sarcomeric genes regulated by LVAD support. Of the sarcomeric genes, troponin-T isoform TnT2 demonstrated the most dramatic change (post-/pre-LVAD = 1.40, $p < 0.001$). Of the sarcomere-associated genes, only calsarcin-1, a calcineurin-binding protein, was down-regulated with LVAD support.

The current study was not designed to differentiate between LVAD-induced changes in ischemic vs non-ischemic cardiomyopathy. However, we did examine the pattern of sarcomeric gene expression in these 2 LVAD groups. Although 3 of 4 patients with idiopathic dilated cardiomyopathy showed a decrease in β-MHC expression after LVAD support, many of the other sarcomeric genes were up-regulated in these samples, and we observed no obvious pattern difference between the ischemic and idiopathic dilated cardiomyopathy LVAD groups. Overall, this global increase in sarcomeric gene expression is consistent with reverse molecular remodeling of the sarcomere.

A possible alternative hypothesis is that apparent gene regulation in pre- vs post- LVAD samples may be caused by regional differences in gene expression, given that these samples were obtained from the left ventricular apex vs the free wall, respectively. To test this, we also evaluated each of the genes in a non-LVAD CHF group of hearts from which both apex and free-wall samples were obtained simultaneously (Figure 1B, Table 2). Of all the sarcomeric genes examined, we found only 1 with greater expression in the free wall than in the apex in the non- LVAD group. For example, β-MHC levels were not significantly different in the free wall than in the apex of the non-LVAD CHF group (Figure 1B), but were greater in the post-LVAD

Table 2. Regulation of Sarcomeric Genes

GenBank#	mRNA	Number of elements on array	Fold change post/pre-LVAD	*p* value	Fold change FW/Apex-CHF	*p* value
X74819	Troponin T, cardiac muscle (TnT2)	2	1.40	<0.001	0.91	0.014
M58018	Cardiac beta-myosin heavy chain (β-MHC)	14	1.32	<0.001	1.00	n.s.
X07897	Troponin C, slow skeletal and cardiac muscles (cTnC)	1	1.31	0.001	0.90	n.s.
M24122	Ventricular myosin alkali light chain (VLC-1)	1	1.23	0.001	0.83	n.s.
AJ010063	Telethonin (titin cap protein)	4	1.15	<0.001	1.03	n.s.
X54163	Troponin I, cardiac muscle (TnI3)	1	1.15	<0.001	0.97	n.s.
M94547	Atrial myosin light chain 2 (ALC-2)	2	1.21	0.002	1.01	n.s.
M21984	Troponin-T beta isoform, fast skeletal muscle (TnT3)	1	1.18	0.005	0.94	n.s.
M12126	Beta-tropomyosin	1	1.08	0.005	0.98	n.s.
M14780	Creatine kinase M chain (M-CK)	1	1.18	0.007	1.22	n.s.
M19308	Troponin T, slow skeletal muscle (TnT1)	1	1.15	0.008	0.97	n.s.
AF111785	Myosin heavy chain IIx/d (MHC IIx/d) (sk.muscle)	1	1.19	0.023	0.99	n.s.
M36172	Atrial embryonic myosin alkali light chain (ALC-1)	2	1.04	0.034	0.88	0.042
M19713	Alpha-tropomyosin (α-TM)	11	1.13	0.036	0.79	<0.001
X51593	Embryonic myosin heavy chain (SMHCE) (sk.muscle)	1	1.13	0.039	1.01	n.s.
X66141	Ventricular myosin light chain 2 (VLC-2)	16	1.14	0.044	0.72	0.062
BC005195	Calcineurin-binding protein calsarcin-1	2	0.90	0.048	0.94	n.s.
M22920	Myosin alkali light chain, smooth muscle (MLC-1sm)	3	1.06	n.s.	0.88	<0.001
X69090	Myomesin 1 (190 kd titin-associated protein)	2	1.12	n.s.	0.84	0.002
X07898	Troponin C, fast skeletal muscle (TnC)	1	1.08	n.s.	0.83	0.008
X90569	Titin	6	1.00	n.s.	1.17	0.017
X54304	Myosin regulatory light chain (MRLC) non-sarcomeric 20 kd	20	0.81	n.s.	0.90	0.070
L47647	Creatine kinase B chain (B-CK)	1	0.96	n.s.	1.17	n.s.

CHF, congestive heart failure; LVAD, left ventricular assist device; FW, free wall; n.s., not significant.

free-wall sample than in the pre-LVAD core (apex) sample (Figure 1A). These findings show, therefore, that the up-regulation of sarcomeric genes observed in patients with LVAD support is not caused by underlying regional differences in expression.

Titin was the single gene that seemed to have greater expression in the non-LVAD free wall vs the apex. However, expression seemed unchanged in the LVAD group, indirectly suggesting that LVAD support might have down-regulated the levels of titin in the ventricle. Several other sarcomeric genes were expressed at lower levels in the free wall vs the apex in the non-LVAD group but seemed unchanged in the LVAD group. This suggests that LVAD support might also have increased the levels of those sarcomeric genes, but these changes may have been masked by regional differences in expression.

Another alternative hypothesis is that apparent gene regulation in pre- vs post-LVAD samples may be caused by changes in the cellular content of the myocardium, particularly because reverse remodeling may involve changes in the fibrous component of this tissue. To test this, we identified cellular markers of cardiofibroblasts, the most prevalent cell type other than cardiomyocytes by hybridizing cDNA from isolated human cardiofibroblasts and cardiomyocytes to the array and then selected genes that had an increased cardiofibroblast-to-cardiomyocyte ratio (Table 3). Not surprisingly, 4 of the most cardiofibroblast-enriched genes were connective tissue growth factor, fibronectin, alpha(III) collagen, and osteonectin, in which

Table 3. Gene Expression Changes of Cardiofibroblast Markers in LVAD and CHF Groups

GenBank#	mRNA	Relative intensity in GM	Relative intensity in CF	CF/CM	Fold change post/pre-LVAD	*p* value	Fold change FW/apex-CHF	*p* value
U14750	Connective tissue growth factor	0.94	51.89	55	1.17	0.045	0.70	0.008
X02761	Fibronectin	1.91	89.76	47	0.79	0.024	0.78	0.003
X06700	Pro-alpha1(III) collagen	0.72	14.36	20	1.18	0.044	0.89	n.s.
J03040	Osteonectin	1.71	30.12	18	1.05	n.s.	0.77	0.006

CHF, congestive heart failure, CF, cardiofibroblasts; CM, cardiomyocytes; FW, free wall; LVAD, left ventricular assist device; n.s., not significant.

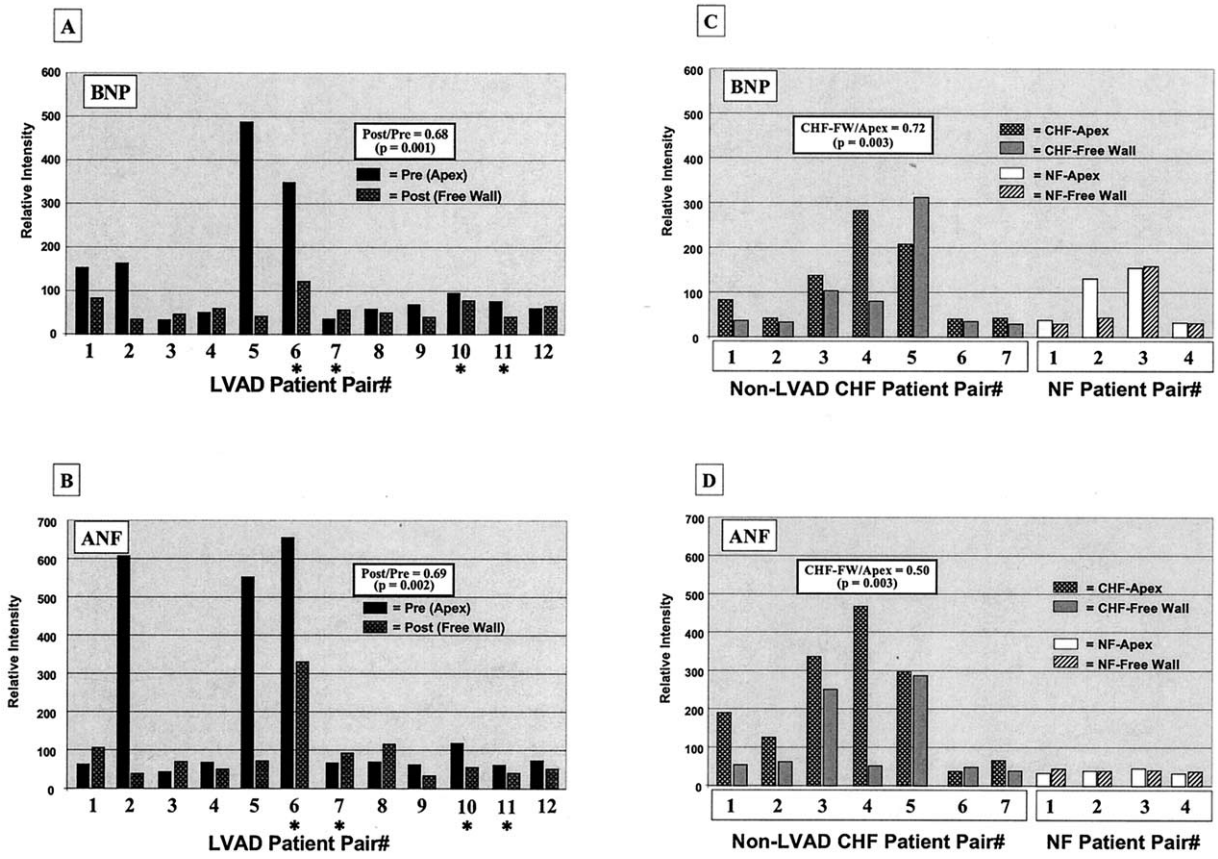

Figure 2. BNP and ANF message levels across individual paired patient samples. Distribution of the relative intensity levels of BNP (A, C) and ANF (B, D) across individual paired patient samples. The graphs represent the relative intensity of a single clone. The fold change and the p value represent the median fold change and the adjusted p value for all clones on the array associated with this gene. *Samples from patients with dilated cardiomyopathy who received LVAD support. ANF, atrial natriuretic factor; BNP, brain natriuretic factor; CHF, congestive heart failure; LVAD, left ventricular assist device; NF, non-failing; post-, after LVAD support; pre-, before LVAD support.

the cardiofibroblast-to-cardiomyocyte ratio was >18. We then evaluated expression of these markers in the LVAD and non-LVAD CHF samples. Connective tissue growth factor, alpha(III) collagen, and osteonectin were up-regulated after LVAD support in all but 1 heart (Patient 1, in whom the fibroblast markers were greater in the pre- vs post-LVAD sample, possibly because of increased fibrous scar in the LVAD insertion site sample, data not shown). Therefore, the observed increase in sarcomeric gene expression was not caused by a decrease in the cardiofibroblast-to-cardiomyocyte content. On the contrary, fibroblast gene expression increased, raising the possibility that the actual magnitude of sarcomeric gene up-regulation may have been even greater than observed in this study.

We also compared the expression of these fibroblast marker genes in the ischemic vs idiopathic dilated cardiomyopathy samples and found no differences in the apparent fibroblast content between the 2 groups of patients (data not shown).

Previous studies have shown that natriuretic proteins and their corresponding mRNAs decrease after LVAD support.[9,19,20] In this study, the mRNA of both brain natriuretic peptide (BNP) and atrial natriuretic factor (ANF) were less in the post-LVAD than in the pre-LVAD samples, but surprisingly they were also less in the free-wall vs the apex of non-LVAD samples (Figure 2), suggesting that neither may have been truly regulated by LVAD support. This regional expression difference was confirmed by slot blot Northern analysis (freewall/apex = 0.39-fold and 0.76-fold for ANF and BNP, respectively, data not shown). The findings for the natriuretic genes contrasts to what was seen for the sarcomeric genes, in which expression did seem to be affected by LVAD support.

DISCUSSION

Previous studies have shown that hemodynamic unloading of the failing heart leads to a decrease in heart size and regression in myocyte hypertrophy,[1,21,22] although 1 study demonstrated a slight increase in myocyte diameter.[2] Histologic changes also have been observed after LVAD support, including improved fiber orientation and

decreased contraction band necrosis.[3] Only a small number of molecular changes that accompany these structural changes are known. In the current study, we examined gene changes caused by LVAD support to gain a better understanding of reverse remodeling. The goal of this study was to find gene-expression changes caused by LVAD support, and although our patient group size is larger than in some previous studies, it is still too small to compare changes in the ischemic vs dilated cardiomyopathy LVAD groups. A total of 17 sarcomeric genes were significantly up-regulated despite a reported decrease in heart size with LVAD support. Because myocyte hypertrophy generally is thought to be decreased by LVAD support, one might expect that total sarcomeric content would decrease. However, the levels of sarcomeric gene expression in this study reflect a change in concentration, not in absolute amount, of sarcomeric mRNA. The increase in the concentration of sarcomeric gene expression was not caused by a decrease in fibroblast content, because fibroblast marker expression levels were not decreased in these samples. In fact, 3 of 4 markers increased, suggesting either an increase in fibroblast content or other participation of fibroblasts in the remodeling process. In either case, this would cause a blunting of the observed regulation of the sarcomeric genes because of the nature of the transcription-profiling methodology. If sarcomere turnover is an integral part of reverse ventricular remodeling, it would be consistent with a need for an increase in relative sarcomeric mRNA content. This fits with previous data, because fiber orientation and the amount of contraction band necrosis have been shown to improve.[3] It is not yet known whether the impact of LVAD support on gene expression is because of improved hemodynamics, or possibly because of alterations in blood flow to the cardiac tissue.

The finding that only 1 sarcomeric-associated gene, calsarcin, was down-regulated during LVAD support potentially is interesting because Frey et al[23] have proposed that calsarcin links calcineurin, a hypertrophic factor, to the contractile apparatus. If calsarcin is involved in the activation of calcineurin, a decrease in calsarcin levels during LVAD support may lead to decreased hypertrophy. The observed decreased hypertrophy seen in reverse remodeling supports this hypothesis.[24]

Contrary to previous reports, our findings suggest that neither ANF nor BNP mRNA levels are regulated by the LVAD-support process. Although differential expression apparently occurs in the pre- (apex) vs post- (free-wall) LVAD samples, we found that this may be attributable to regional differences in the expression of these genes, rather than to true regulation in response to LVAD support. However, these results are not necessarily inconsistent with reported decreases in serum ANF and BNP protein as a result of LVAD support[25] because ANF and BNP are stored in regulated secretory granules and are released on stimulation.[26,27] It would not be surprising that the serum levels of proteins released from the regulated secretory pathway are at best loosely associated with their message levels. Given the extremely variable levels of expression of natriuretic peptides among patients and within regions of the heart, generalizations may not be possible.

CONCLUSIONS

Left ventricular assist device support has been shown to result in increased cardiac performance[5] and increased contractility in isolated myocytes.[4] The increased performance may result from the reverse remodeling process, which currently is poorly understood. During the preparation of this manuscript, a report by Blaxall et al[28] demonstrated changes in a number of genes pre- vs post-LVAD support, including changes in genes involved in metabolism. Because of the difference in content of the arrays, it is difficult to compare the studies. Our array was smaller in gene number, but was custom-built for cardiac discovery and thus may have allowed us to detect a global change in sarcomeric genes. We found significant increases in the expression of numerous contractile proteins as well as the known changes in calcium-handling genes. We also noted the down-regulation of a gene that may be associated with the initial hypertrophic response to heart failure. Our control experiments show that none of these observed changes are caused by regional differences in expression or by differences in cellular content of the samples. In fact, the apparent modest yet significant changes in the numerous sarcomeric proteins may have been blunted by a slight increase in fibroblast gene expression. The current study indicates that the reverse remodeling of the heart associated with LVAD support is accompanied by molecular changes in the contractile apparatus and that these changes are consistent with improved contractile band morphology[3] and cardiac contractility.[4]

LIMITATIONS

The patients enrolled in this study were heterogeneous in genetic background, environment, and medical treatment. We attempted to control for some of the variability by restricting our comparisons to paired samples from individuals. We also attempted to control for regional differences in expression in the left ventricle using samples from patients with heart failure who were not subjected to LVAD support. However, these patients are not as hemodynamically compromised as are patients with LVAD support, and this may affect gene expression in different regions of the heart. Also, as with all LVAD studies, medications necessarily were different before and during LVAD support.

The authors thank Nancy Stagliano, Tina Thorne, Kristine Israelian, Attilio Rigotti, Trudy Wilson, Jean Courtemanche, Hugues Bernard, and members of the Transcription Profiling Core Group for assistance.

REFERENCES

1. Levin HR, Oz MC, Chen JM, Packer M, Rose EA, Burkhoff D. Reversal of chronic ventricular dilation in patients with end-stage cardiomyopathy by prolonged mechanical unloading. Circulation 1995;91:2717–20.
2. McCarthy PM, Nakatani S, Vargo R, et al. Structural and left ventricular histologic changes after implantable LVAD insertion. Ann Thorac Surg 1995;59:609–13.
3. Nakatani S, McCarthy PM, Kottke-Marchant K, et al. Left ventricular echocardiographic and histologic changes: impact of chronic unloading by an implantable ventricular assist device. J Am Coll Cardiol 1996;15:894–901.
4. Dipla K, Mattiello JA, Jeevanandam V, Houser SR, Margulies KB. Myocyte recovery after mechanical circulatory support in humans with end-stage heart failure. Circulation 1998;97:2316–22.
5. Heerdt PM, Holmes JW, Cai B, et al. Chronic unloading by left ventricular assist device reverses contractile dysfunction and alters gene expression in end-stage heart failure. Circulation 2000;102:2713–9.
6. James KB, McCarthy PM, Thomas JD, et al. Effect of the implantable left ventricular assist device on neuroendocrine activation in heart failure. Circulation 1995; 92(suppl 2):191–5.
7. Torre-Amione G, Stetson SJ, Youker KA, et al. Decreased expression of tumor necrosis factor-alpha in failing human myocardium after mechanical circulatory support: a potential mechanism for cardiac recovery. Circulation 1999;100:1189–93.
8. Birks E, Latif N, Owen V, et al. Quantitative myocardial cytokine expression and activation of the apoptotic pathway in patients who require left ventricular assist devices. Circulation 2001;104(suppl 1):233–40.
9. Altemose GT, Gritsus V, Jeevanandam V, Goldman B, Margulies KB. Altered myocardial phenotype after mechanical support in human beings with advanced cardiomyopathy. J Heart Lung Transplant 1997;16:765–73.
10. Estrada-Quintero T, Uretsky BF, Murali S, Griffith BP, Kormos RL. Neurohormonal activation and exercise function in patients with severe heart failure and patients with left ventricular assist system. Chest 1995;107:1499–503.
11. Madigan JD, Barbone A, Choudhri AF, et al. Time course of reverse remodeling of the left ventricle during support with a left ventricular assist device. J Thorac Cardiovasc Surg 2001;121:902–8.
12. Takeishi Y, Jalili T, Hoit BD, et al. Alterations in Ca2+ cycling proteins and Gαq signaling after left ventricular assist device support in failing human hearts. Cardiovasc Res 2000;45:883–8.
13. Ogletree-Hughes ML, Stull LB, Sweet WE, Smedira NG, McCarthy PM, Moravec CS. Mechanical unloading restores beta-adrenergic responsiveness and reverses receptor downregulation in the failing human heart. Circulation 2001;104:881–6.
14. Li YY, Feng Y, McTiernan CF, et al. Downregulation of matrix metalloproteinases and reduction in collagen damage in the failing human heart after support with left ventricular assist devices. Circulation 2001;104:1147–52.
15. Frazier OH. First use of an untethered, vented electric left ventricular assist device for long-term support. Circulation 1994;89:2908–14.
16. Dipla K, Mattiello JA, Margulies KB, Jeevanandam V, Houser SR. The sarcomeric reticulum and the Na+/Ca2+ exchanger both contribute to the Ca2+ transient of failing human ventricular myocytes. Circ Res 1999;84: 435–44.
17. Chiang LW, Grenier JM, Ettwiller L, et al. An orchestrated gene expression component of neuronal programmed cell death revealed by cDNA array analysis. Proc Natl Acad Sci U S A 2001;98:2814–9.
18. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A 2001;98:5116–21.
19. Morawietz H, Szibor M, Goettsch W, et al. Deloading of the left ventricle by ventricular assist device normalizes increased expression of endothelin ET(A) receptors but not endothelin-converting enzyme-1 in patients with end-stage heart failure. Circulation 2000;102:III188–93.
20. Bartling B, Milting H, Schumann H, et al. Myocardial gene expression of regulators of myocyte apoptosis and myocyte calcium homeostasis during hemodynamic unloading by ventricular assist devices in patients with end-stage heart failure. Circulation 1999;100:II216–23.
21. Scheinin SA, Capek P, Radovancevic B, Duncan JM, McAllister HA, Jr, Frazier OH. The effect of prolonged left ventricular support on myocardial histopathology in patients with end-stage cardiomyopathy. ASAIO J 1992;38:M271–4.
22. Jacquet L, Zerbe T, Stein KL, Kormos RL, Griffith BP. Evolution of human cardiac myocyte dimension during prolonged mechanical support. J Thorac Cardiovasc Surg 1991;101:256–9.
23. Frey N, Richardson JA, Olson EN. Calsarcins, a novel family of sarcomeric calcineurin- binding proteins. Proc Natl Acad Sci U S A 2000;97:14632–7.
24. Zafeiridis A, Jeevanandam V, Houser SR, Margulies KB. Regression of cellular hypertrophy after left ventricular assist device support. Circulation 1998;98:656–62.
25. Sodian R, Loebe M, Schmitt C, et al. Decreased plasma concentration of brain natriuretic peptide as a potential indicator of cardiac recovery in patients supported by mechanical circulatory assist systems. J Am Coll Cardiol 2001;38:1942–9.
26. Canaff L, Brechler V, Reudelhuber TL, Thibault G. Secretory granule targeting of atrial natriuretic peptide correlates with its calcium-mediated aggregation. Proc Natl Acad Sci U S A 1996;93:9483–7.
27. Bialik GM, Abassi ZA, Hammel I, Winaver J, Lewinson D. Evaluation of atrial natriuretic peptide and brain natriuretic peptide in atrial granules of rats with experimental congestive heart failure. J Histochem Cytochem 2001;49:1293–300.
28. Blaxall BC, Tschannen-Moran BM, Milano CA, Koch WJ. Differential gene expression and genomic patient stratification following left ventricular assist device support. J Am Coll Cardiol 2003;4:1096–106.